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NEUROPROTECTION WITH TICLOPIDIN AND OTHER INHIBITORS OF PLATELET AGGREGATION

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Treatment with inhibitors of platelet aggregation is established for secondary stroke prevention. Inhibition of energy metabolism was suggested as a possible mechanism for inhibition of platelet aggregation. Mild inhibition of energy metabolism can increase tolerance against hypoxia .The goal of the present study was to investigate whether pretreatment with inhibitors of platelet aggregation I increases cellular tolerance against hypoxia.

Hippocampal slices were prepared from male CD-1 mice. Pretreatment was achieved by a single i.p. injection (20 mg/kg body weight) of ticlopidin or clopidogrel 6 hours before slice preparation. Population spikes were recorded in hippocampal region CA-1 upon stimulation of Schaffer collaterals. Upon 15 min of baseline recording with oxygenated Ringer's solution the recording chamber was perfused with hypoxic Ringer's solution bubbled with 95%N_2 and 5% CO_2. After 15 min of hypoxia slices were superfused with oxygenated Ringer's solution until end of experiment.

Posthypoxic recovery of population spike amplitude in control slices was 33±23%. After pretreatment with acetysalicyclic acid posthypoxic population spike amplitude (PSAP) was increased to 75±36% PSAP after pretreatment with ticlopidin was 61±48% .In slices pretreated with clopidogrel PSAP was 68 ±38% .We conclude that pretreatment with inhibitors of platelet aggregation acetylsalicylic acid, ticlopidin and clopidogrel increases cellular tolerance against hypoxia.

Supported by a grant of the University of Ulm to M.W.Riepe.

235.22

EFFECTS OF TTC-909 ON CEREBRAL INFARCTION FOLLOWING PERMANENT OCCLUSION AND PHOTOCHEMICALLY-INDUCED THROMBOSIS OF MIDDLE CEREBRAL ARTERY IN STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS.

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It was previously shown that TTC-909, a chemically stable prostacyclin analogue referred to as clinprost incorporated into lipid microspheres, improved changes in microcirculation and glucose utilization following focal cerebral ischemia in stroke-prone spontaneously hypertensive rats (SHRSP). In this study, we examined the effects of TTC-909 on the infarct size after permanent occlusion and photochemically-induced thrombosis (PIT) of middle cerebral artery (MCA) in SHRSP. In the permanent MCA occlusion model, the schedule of treatment with TTC-909 was as follows; (1) bolus injection once daily over 7 days starting immediately after MCA occlusion, (2) infusion for 3 hours starting immediately after MCA occlusion, (3) infusion for 3 hours starting immediately after MCA occlusion followed by bolus injection once daily over 6 days. The cerebral infarction was limited to cerebral cortex at 7 days after permanent MCA occlusion. When TTC-909 was infused for 3 hours starting immediately after MCA occlusion followed by bolus injection, the infarct volume was reduced dose-dependently. In the other groups treated with TTC-909 ((1)(2)), the infarct volume was not reduced. In the PIT-MCA occlusion model, the cerebral infarction was recognized from cerebral cortex to striatum 24 hours after thrombotic MCA occlusion. When TTC-909 in doses of 100, 300 and 900 ng/kg/hr was intravenously infused for 3 hours starting immediately after thrombotic MCA occlusion, the infarct volume was reduced dose-dependently. These results suggest that cerebral infarction induced by MCA occlusion in SHRSP is progressive, and that TTC-909 is effective in both permanent and thrombotic focal ischemia models in SHRSP. TTC-909 may have the efficacy in the acute phase of cerebral infarction.

ISCHEMIA: NEUROPROTECTION III

236.1

POTENT σ_1-RECEPTOR LIGAND, PPBP [4-PHENYL-1-(4-PHENYLBUTYL) PIPERIDINE] AFFORDS NEUROPROTECTION FROM FOCAL ISCHEMIA AND PROLONGED REPERFUSION

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We have previously shown that intravenous administration of the potent sigma (σ_1) receptor ligand 4-phenyl-1-(4-phenylbutyl)piperidine (PPBP) provides neuroprotection against transient focal cerebral ischemia which is dependent on treatment duration. We tested the hypothesis that PPBP would provide neuroprotection in a model of transient focal ischemia and 7 days of reperfusion in the rat as assessed by neurobehavioral outcome and infarction volume. Under controlled conditions of normoxia, normocarbia and normothermia, halothane-anesthetized male Wistar rats were subjected to 2 hours of middle cerebral artery occlusion (MCAO) by the intraluminal occlusion using laser Doppler flowmetry. Sixty minutes following the onset of ischemia, rats were randomly assigned to 4 treatment groups in a blinded fashion to receive continuous intravenous infusion of control saline or 0.1, 1 or 10 µmol/Kg/hr PPBP for 24 hours. Neurobehavioral evaluation was performed at baseline (3-4 days prior to MCAO), 3 and 7 days of reperfusion. Infarction volume was assessed by triphenyltetrazolium chloride (TTC) staining on day 7 of reperfusion in all rats. TTC-determined infarction volume (corrected for edema) of ipsilateral hemisphere was smaller in rats treated with 10 µmol/Kg/hr PPBP (123±20 mm^3; 17±3% of ipsilateral hemisphere; $p<0.05$) (mean±SEM) as compared to corresponding rats treated with saline. Although MCAO was associated with several alterations in neurobehavior, treatment with PPBP did not have an effect on behavioral outcomes. These data demonstrate that the potent σ_1 receptor ligand PPBP decreases infarction volume without altering neurobehavior following transient focal ischemia and prolonged reperfusion in the rat. *Supported by USPHS NIH Grant NS20020 and AHA*

236.2

PREVENTION OF SELECTIVE NEURONAL DEATH BY MAGNESIUM SULPHATE FOLLOWING TRANSIENT CEREBRAL ISCHEMIA IN THE RAT.

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Rats were exposed to 8 minutes of transient cerebral ischemia, an insult resulting in the selective death of hippocampal CA1 neurons. Initial experiments determined the pre-ischemic dose of an intravenous (IV) infusion of magnesium sulphate 7-hydrate ($MgSO_4$) which provided maximal neuroprotection. Animals (n=5/group) received either a 90 mg/kg IV loading dose (LD) of $MgSO_4$ alone or an IV LD of 90 mg/kg followed by a 48 hour IV infusion of $MgSO_4$ at either 15, 30, 60 or 120 mg/kg/hr. Animals which received the LD alone, showed 30% neuronal preservation at 7 days post-ischemia as compared to the control groups; non-ischaemic (100%), sham operated (100%), untreated ischaemic (<5%), vehicle-treated (<5%; normal saline) ischemic rats. Animals which received the LD followed by a continuous IV infusion of $MgSO_4$ at either 15, 30, 60 or 120 mg/kg/hr showed 30%, 80%, 15% and <5% neuronal preservation, respectively. The 90 mg/kg LD and 30 mg/kg/hr IV infusion was then administered 4, 8, 12 or 24 hours after ischemia and resulted in 80%, 70%, 55% and 35% neuronal preservation, respectively.

These results highlight: (i) a previously undocumented $MgSO_4$ dose response, with higher doses being ineffective; and (ii) the neuroprotective potential for pre- and post-ischemic IV administration of $MgSO_4$.

Supported by ANRI/SCGH and RACS.

236.3

INTRAVENTRICULAR INFUSION OF APOLIPOPROTEIN E (APOE) REDUCES NEURONAL DAMAGE IN APOE DEFICIENT MICE FOLLOWING GLOBAL ISCHEMIA.

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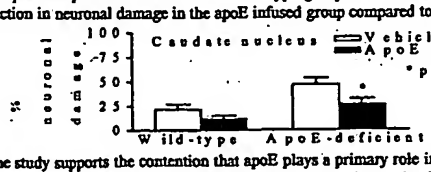
Apolipoprotein E (apoE) plays a role in brain injury. We have shown that apoE deficient mice have significantly increased neuronal damage following global ischemia. The objective of this study was to test our hypothesis that intraventricular infusion of apoE would reduce neuronal damage following global ischemic injury.

ApoE-deficient and wild-type mice (16 week old males, n=12 per treatment group) were anesthetised with halothane. A canmula was placed stereotactically into the right lateral ventricle via which apoE 5mg/ml or vehicle was infused (1ml/hr, for 72hr). Transient global ischemia was induced by bilateral carotid artery occlusion for 17min. 72hr later, the brains were perfusion fixed and sections cut and stained with H&E. The percentage of ischemic neurons in the caudate nucleus of the vehicle infused group was compared to the apoE infused group using Students t-test. There was statistically less ischemic neuronal damage in the apoE-deficient mice infused with apoE compared to vehicle. In the wild-type group there was a trend towards a reduction in neuronal damage in the apoE infused group compared to vehicle.



The study supports the contention that apoE plays a primary role in the response to acute brain injury and may be used as a potential therapy in treating brain injury.

This work was supported by The Wellcome Trust (K. Horsburgh, J.A.R. Nicoll).

236.4

ISCHEMIC SEVERITY AND NEUROPROTECTION BY CDP-CHOLINE.

J. F. Hatcher, R. J. Dempsey* and A. M. Rao. Dept. of Neurological Surgery, Univ. of Wisconsin, Madison, WI 53792.

CDP-choline (CDPC) is a rate-limiting intermediate in the biosynthesis of phosphatidylcholine (PC), an important component of the neuronal membrane. The ability of CDPC to alter phospholipid metabolism has been shown to be an important function in experimental ischemia. Exogenous treatment with CDPC stimulates PC synthesis and prevents release of free fatty acids (FFA) especially arachidonic acid (AA) after ischemia/reperfusion. AA is one of the risk factors directly acting on neurons and its accumulation is greatest in vulnerable brain regions in ischemic injury. Here we report the neuroprotective effect of CDPC in hippocampal CA_1 region after transient ischemia of gerbils. Results: Ischemia-reperfusion resulted in AA and leukotriene C_4 accumulation, blood-brain barrier dysfunction, and edema; CDPC significantly attenuated all of these parameters. 5- or 10-min global ischemia followed by 6-d reperfusion resulted in 88±7% neuronal loss in the CA_1 subfield of the hippocampus. CDPC (500mg/kg i.p.) administered just after 5-min ischemia and thereafter daily for 5 d significantly attenuated neuronal death to 12±4% ($p<0.05$ compared to untreated ischemic group). When ischemic duration was increased to 10-min, CDPC also significantly decreased neuronal death to 31±6% ($p<0.05$ compared to untreated ischemic group). CDPC administered as one dose (immediately after ischemia) did not provide significant protection, whereas two doses (at 0 and 1-d) provided slight neuroprotection. Conclusions: Accumulated AA after transient ischemia is converted to oxygenated metabolites through cyclooxygenase/lipoxygenase pathways, which are involved in BBB dysfunction, edema and neuronal loss. CDPC may protect neurons by preventing the membrane PC breakdown into AA and subsequent free radical-generating metabolites.

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ABSTRACTS

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Part 1

PART 1 OF 2

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